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FACTORS AFFECTING PATHOGENICITY OF PINK ROOT FUNGUS OF ONIONS [1]

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INTRODUCTION

In a previous paper (*2*) [3] a study of a number of isolates of the causal fungus of pink root of onion (*Allium cepa* L.) was reported. It was shown that several strains produced pycnidia in culture and that others were completely sterile. Mutants that were sterile appeared regularly in old cultures. All fertile isolates produced pycnidia on roots or cotyledons of onions grown in inoculated quartz sand supplied with a balanced nutrient solution, but only one isolate, which had been single-spored twice in succession, produced pycnidia on the roots of plants grown in inoculated soil. The pycnidia of these fertile isolates were always found to bear setae, and, on the basis of this hitherto unreported fact, the fungus was transferred from *Phoma terrestris* Hansen to *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, and Larson.

The importance of the development of varieties of onion resistant to pink root for use in areas where the disease is a limiting factor has been emphasized by Porter and Jones (*5*). A method was needed whereby large numbers of seedlings could be tested rapidly under reproducible, controlled conditions and various isolates of *Pyrenochaeta terrestris* could be used as test pathogens. In the development of such a method studies were carried out on the physiology of the fungus, on its variability in pathogenicity, and on the relation of environal factors to the development of the disease in young seedlings. When optimum conditions had been determined, the relative resistance of a series of standard varieties was found by the inoculation of seedlings. The results of the investigations on physiology, pathogenicity, and host resistance are presented in this paper.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 18.

METHODS AND MATERIALS

The isolates of *Pyrenochaeta terrestris*, their sporulating capacity, and their sources are listed in table 1. As a rule the strains or varieties of onions to be tested were grown from seed, but in a few cases sets were used.

TABLE 1.—*Key numbers, sources, and sporulating capacity of isolates of Pyrenochaeta terrestris used*

Key No.[1]	Source	Type of original material	Sporulation of isolates on culture medium	Collector
Cal-B-H	Berkeley, Calif.	Culture	−	H. N. Hansen.
Cal-D-H	Davis, Calif.	Soil	−	G. N. Davis
Cal-D-T	do	Roots	+	L. D. Leach.
Col-RF-T	Rocky Ford, Colo.	Soil	−	A. M. Binkley.
Col-FC-T	Fort Collins, Colo.	do	−	Do.
Col-FC1-T	do	do	+	Do.
Col-FC2-T	do	do	−	Do.
Col-FC-H	do	do	−	Do.
La-S	Baton Rouge, La.	Roots	+	F. J. LeBeau.
La-S-S	do	do	+	Do.
La-M-T	do	do	−	Do.
Tex-H	Winter Haven, Tex.	do	−	S. S. Ivanoff.
Tex-T	do	do	+	B. A. Perry.
Tex-1-S	do	do	−	Do.
Tex-2-S	do	do	−	Do.
Tex-1-H	do	do	−	Do.
Tex-1-T	do	do	−	Do.
Iowa-H	Clear Lake, Iowa	Soil	−	F. S. Haber.
Iowa-1-T	Fertile, Iowa	Roots	−	W. J. Hooker.
Iowa-S	do	do	+	Do.
Iowa-T	do	do	+	Do.
Utah-O-H	Utah	Soil	−	
Utah-H	do	do	−	
Utah-T	do	do	−	
Ill-T	Burlington, Ill.	Roots	+	A. M. Gorenz.
Mich-H	East Lansing, Mich.	Culture	−	A. L. Andersen.
Mass-T	Amherst, Mass.	Roots	−	H. M. Yegian.
Wis-M-T	Madison, Wis.	do	−	A. M. Gorenz.
Wis-R-T	Racine, Wis.	do	−	Do.

[1] H indicates a hyphal-tip transfer; S, a single-spore transfer; T, a mass transfer.

Clean, fine, white quartz sand was used as the host substrate in most instances. In some experiments it was contained in galvanized-iron pans, 3.5 inches deep, 12 inches wide, and 22 inches long, placed in Wisconsin soil-temperature tanks (fig. 1) in a greenhouse in which the temperature ranged for the most part from 20° to 24° C. In other experiments sand cultures were run in 6-inch crocks fitted with siphons as described by Schroeder and Walker (*7*). In the study of the relation of hydrogen-ion concentration of the nutrient to disease development, a sand culture with a continuous drip of nutrient, as described by Pryor (*6*), was used.

To prepare inoculum, transfers were made from stock cultures of *Pyrenochaeta terrestris* to petri dishes containing Czapek's dextrose-

malt agar [4] and kept at 28° C. for 6 days. Nine 6-mm. disks from the border of the fungus mats in the petri dishes were transferred to 300 cc. of Czapek's modified liquid medium [5] in 32-ounce bottles, which were incubated in a horizontal position for 10 days at 28° with daily shaking to insure adequate aeration. The fungus and medium were macerated in a Waring Blendor for 3 minutes, and 100 cc. of this inoculum (except when a different amount is indicated) diluted with 900 cc. of distilled water was mixed with 10 kg. of sand.

When pans were used, the inoculated sand formed a layer approximately 2.5 inches deep, in which were marked 8 furrows one-half inch deep. Each furrow was divided into 4 equal sections, or 32 sections per pan. Two pans were inoculated with each isolate. Each half pan was considered a replicate and 16 varieties were planted

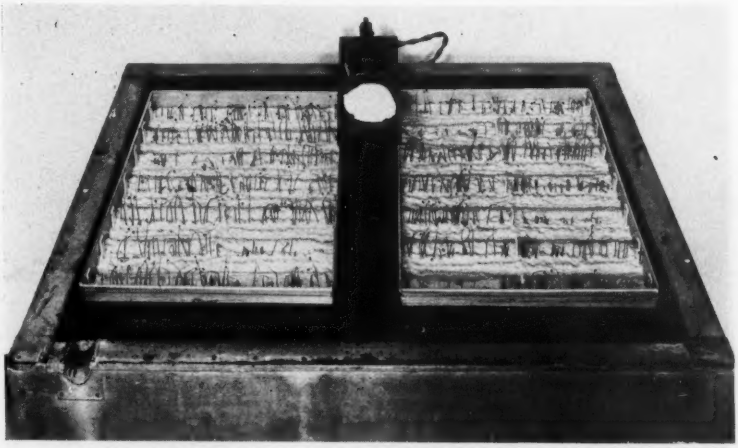

FIGURE 1.—Individual Wisconsin soil-temperature tank with culture pans.

at random in 4 replicates, so that the reliability of the data could be checked by statistical analysis. Approximately 30 seeds were planted in each section. After the seeds were planted, the pans were placed at random in the soil-temperature tanks and were covered with cardboard for 7 or 8 days; at that time the seedlings were about 1 inch high. The pans were then flooded with distilled water, the excess was siphoned off, and the pans were rewatered with the modified Hoagland's solution [6] used by Walker and Hooker (*9*) and designated by them as 1H. Subsequently, until the end of the experiment, the pans were watered as needed with distilled water. In figure the

[4] 2 gm., $NaNO_3$; 1 gm., KH_2PO_4; 0.5 gm., KCl; 0.5 gm., $MgSO_4$; 0.01 gm., $FeSO_4$; 20 gm., dextrose; 15 gm., malt extract; 20 gm., agar; 1,000 cc., water.

[5] 2 gm., $NaNO_3$; 1 gm., KH_2PO_4; 0.5 gm., KCl; 0.05 gm., $MgSO_4$; 0.01 gm., $FeSO_4$; 50 gm., dextrose; 15 gm., malt extract; 1,000 cc., water.

[6] The formula used was as follows: $Ca(NO_3)_2$, 0.005M; KNO_3, 0.005M; $MgSO_4$, 0.002M; KH_2PO_4, 0.001M; NaCl, 0.001M; traces of H_3BO_3, $CuCl_2$, $ZnCl_2$, $MnCl_2$, $FeCl_3$.

FIGURE 2.—Sand-culture pans arranged in a series of Wisconsin soil-temperature tanks.

general arrangement of the culture pans in the soil-temperature tanks and the siphon in the corner of each pan are shown.

The seedlings were carefully removed from the sand 28 to 30 days after planting and graded as to severity of symptoms of pink root into five disease classes, each of which was given a class weight as follows: Free of pink root, 0; slightly affected, 25; moderately affected, 50; severely affected, 75; dead, 100. A disease index for each lot was obtained by multiplying the number of plants in each class by the class weight, adding the products, and dividing the sum by the total number of plants.

EXPERIMENTAL RESULTS

CONCENTRATION OF INOCULUM

Inoculum of isolate Utah–T of *Pyrenochaeta terrestris* was mixed at the rate of 12.5, 25, 50, 100, or 200 cc. to 10 kg. of sand. The inoculated substrate was leveled off to within 1.5 inches of the top of 6-inch crocks. Each concentration of inoculum was replicated four times at 16°, 20°, 24°, and 28° C. in Wisconsin soil-temperature tanks. Excel, Texas Grano, Mountain Danvers, and Yellow Globe Danvers varieties were planted at the rate of 30 seeds of each variety. Data were taken 28 days after planting (table 2).

TABLE 2.—*Concentration of inoculum of isolate Utah–T of Pyrenochaeta terrestris in relation to disease development on 4 onion varieties at 4 sand temperatures*

Variety	Concentration	Disease index at temperature indicated			
		16° C.	20° C.	24° C.	28° C.
	Cc. per 10 kg.				
Excel	12.5	0	3	16	24
	25.0	0	4	28	39
	50.0	1	10	27	72
	100.0	4	19	38	76
	200.0	5	20	40	71
Texas Grano	12.5	0	10	70	96
	25.0	2	36	89	96
	50.0	14	41	84	100
	100.0	5	28	80	99
	200.0	10	56	95	99
Mountain Danvers	12.5	0	18	87	100
	25.0	6	55	97	98
	50.0	14	49	93	100
	100.0	11	54	94	100
	200.0	16	51	96	100
Yellow Globe Danvers	12.5	0	3	67	96
	25.0	0	15	87	91
	50.0	5	23	85	98
	100.0	6	28	75	98
	200.0	8	28	89	100

The disease index was influenced by resistance of the host, the temperature of the substrate, and the concentration of the inoculum. At 16° C. the disease was so slight that the influences of variety and concentration of inoculum were not measurable. The indices were higher at 20° than at 16°. There was a steady increase of the index of the most resistant variety, Excel, with increase of inoculum, but the index curves of the other three varieties, which were obviously

more susceptible than Excel, leveled off at 50 cc. or less of inoculum. At the more favorable temperatures, 24° and 28°, the differential in index due to inoculum became very slight or nil except in Excel. At 28° the indices of this variety were lower at all concentrations than those of the other varieties, but concentrations of 50, 100, and 200 cc. of inoculum did not have appreciably different effects on Excel. It was concluded that the maximum index due to increase in concentration of inoculum was reached more promptly as the temperature increased and as the inherent resistance of the host decreased. The relations of concentration of inoculum and of temperature to the disease indices of the most resistant variety, Excel, and of one of the three more susceptible varieties, Yellow Globe Danvers, are shown graphically in figure 3.

VARIATION IN PATHOGENICITY OF ISOLATES

Inocula of isolates Utah–H, Mich–H, Iowa–H, Tex–H, Cal–B–H, and Cal–D–H were prepared in the usual manner, and 6-inch crocks of sand and of soil (two-thirds greenhouse loam, one-third muck soil) were inoculated. Five White Portugal sets, surface-sterilized in 1 : 1,000 mercuric chloride for 20 minutes, were planted in each crock. The crocks were then placed in soil-temperature tanks set at 25° C. Plants were pulled after 30 days and rated as to severity of infection (table 3). Isolates Utah–H, Tex–H, and Cal–D–H were highly pathogenic, isolates Cal–B–H and Iowa–H moderately so, and isolate Mich–H weakly pathogenic in both sand and soil. In general a lower disease rating was obtained in soil than in sand.

TABLE 3.—*Pathogenicity of 6 isolates of Pyrenochaeta terrestris on White Portugal onion sets in sand and soil at 25° C.*

Isolate	Disease index in—		Isolate	Disease index in—	
	Sand	Soil		Sand	Soil
Utah–H	100	100	Cal–B–H	40	5
Tex–H	100	90	Iowa–H	45	5
Cal–D–H	100	80	Mich–H	5	0

In another experiment a second group of isolates was tested on seedlings in sand culture in pans. Two rows each of Yellow Bermuda, the parent of Excel (table 2), and Southport Yellow Globe varieties were planted in each of two pans inoculated with each isolate. The isolates used and the disease indices obtained 28 days after planting at 24° C. are presented in table 4. The disease indices for Southport Yellow Globe ranged from 100 for isolate La–S to 44 for isolate Wis–M–T. The indices for Yellow Bermuda were lower than those for Southport Yellow Globe. For any isolate the differences between variety indices were highly significant. The descending order of the indices was not the same for Yellow Bermuda as for Southport Yellow Globe. However, isolates La–S and Tex–T were more virulent than the others and Wis–M–T was the least virulent on both varieties.

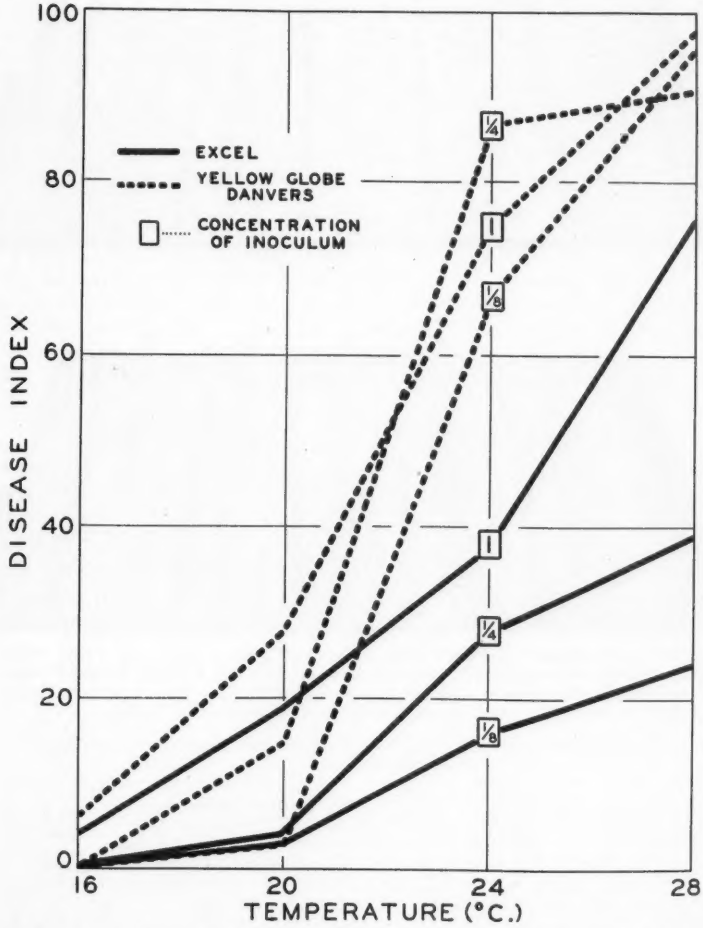

FIGURE 3.—Relation of temperature and concentration of inoculum to disease indices in seedlings of Excel and Yellow Globe Danvers onions. 1,100 cc. of inoculum to 10 kg. of sand; ¼, 25 cc.; ⅛, 12.5 cc.

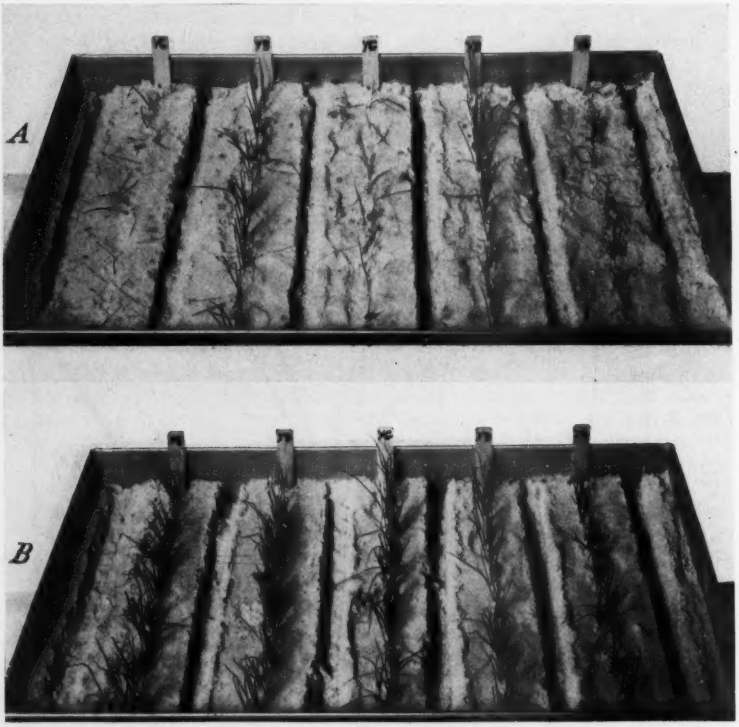

FIGURE 4.—Southport Yellow Globe (YG) and Yellow Bermuda (YB) plants grown for 28 days at 24° C. in sand inoculated with (*A*) a highly virulent isolate (La–S) and (*B*) a weakly virulent isolate (Col–FC–T). The greater resistance of Yellow Bermuda plants is more evident with isolate La–S.

TABLE 4.—*Pathogenicity of 8 isolates of Pyrenochaeta terrestris on seedlings of 2 onion varieties at 24° C.*[1]

Isolate	Disease index in variety—		Isolate mean
	Southport Yellow Globe	Yellow Bermuda	
La–S	100	90	95.0
Tex–T	99	91	95.0
Utah–T	88	48	68.0
Col–RF–T	86	39	62.5
Mass–T	76	64	70.0
Col–FC1–T	75	39	57.0
Iowa–T	73	48	60.5
Wis–M–T	44	29	36.5
Variety mean	80.1	56.0	

[1] Least significant difference:

	5-percent level	*1-percent level*
Varieties	1.7	2.3
Isolates	3.5	4.6
Varieties × isolates	6.9	9.2
Within varieties or isolates	4.9	6.5

The comparative studies of isolates showed that rather wide differences in virulence existed. Other experiments, not presented in detail, showed that isolates from the same locality also varied in virulence. From the comparison of host varieties it appeared that the differences in host resistance were less pronounced when highly virulent isolates were used as the source of inoculum than when weakly virulent isolates were used. A comparison of Yellow Bermuda and Southport Yellow Globe varieties infected by a highly virulent isolate and a weakly virulent one is given in figure 4. Insofar as observations were conducted, cultures of individual isolates maintained by mass transfers on culture media about every 3 months retained about the same degree of virulence for 18 months.

TEMPERATURE RELATIONS

Hansen (*3*) found the optimum for growth of the pink root fungus on corn-meal agar to be about 26° C. Davis and Henderson (*1*) reported the optimum for growth on potato-dextrose agar to be 28°. In this investigation, plates containing 25 cc. of potato-dextrose agar were inoculated with 6-mm. disks from the borders of colonies on the same kind of agar. The isolates studied and the average diameters of colonies at 8 and 12 days are given in table 5. Six isolates made optimum growth at 28°, while two showed best growth at 24° on both the eighth and the twelfth day. Three (Utah–O–H, Mass–T, and Cal–D–T) made about as good growth at 24° as at 28°. The rates of increase of colony diameters with increases of temperature were not the same for all isolates. This is shown graphically in figure 5. The least growth at 28° was made by Mass–T and the most by Wis–R–T; the growth of Tex–1–H was intermediate.

TABLE 5.—*Relation of temperature to growth of 11 isolates of Pyrenochaeta terrestris on potato-dextrose agar*

Isolate	Diameter of colonies at temperature and days indicated [1]									
	16° C.		20° C.		24° C.		28° C.		32° C.	
	8	12	8	12	8	12	8	12	8	12
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
Utah–O–H	17.5	31.8	29.5	41.5	33.5	56.5	33.0	58.0	16.8	35.3
La–M–T	14.3	29.5	28.5	55.8	45.8	78.8	47.8	90.0	24.8	44.8
Ill–T	17.0	34.0	32.0	55.8	45.3	71.8	40.0	64.0	22.0	43.0
La–S–S	14.3	27.5	25.0	49.0	47.0	78.8	42.5	72.5	21.0	41.0
Iowa–T	14.5	26.3	25.3	46.0	44.0	71.0	51.0	90.0	19.8	33.5
Col–FC–H	11.5	27.0	23.0	49.5	44.8	76.0	48.5	90.0	29.5	46.5
Tex–1–H	17.0	26.5	27.8	48.8	37.5	63.3	43.8	68.5	6.3	10.0
Wis–R–T	11.8	20.5	31.0	54.5	48.5	84.0	55.8	90.0	15.0	19.0
Wis–M–T	18.8	29.0	37.3	59.8	46.0	72.0	53.0	72.3	16.5	25.3
Mass–T	11.0	20.5	25.3	44.5	30.8	44.5	28.0	51.5	2.0	3.8
Cal–D–T	12.5	27.0	25.5	49.7	46.5	76.8	47.5	71.3	31.3	48.0

[1] At a reading of 90.0 the plate was completely occupied by the culture.

The relation of temperature to development of pink root was investigated by Hansen (*3*). Hansen planted onion bulbs in inoculated steam-sterlilized soil in pots which were placed at 4° C., 10°, 13°, 20°, 25°, or 30° in the dark. He found that all of the 20 plants exposed

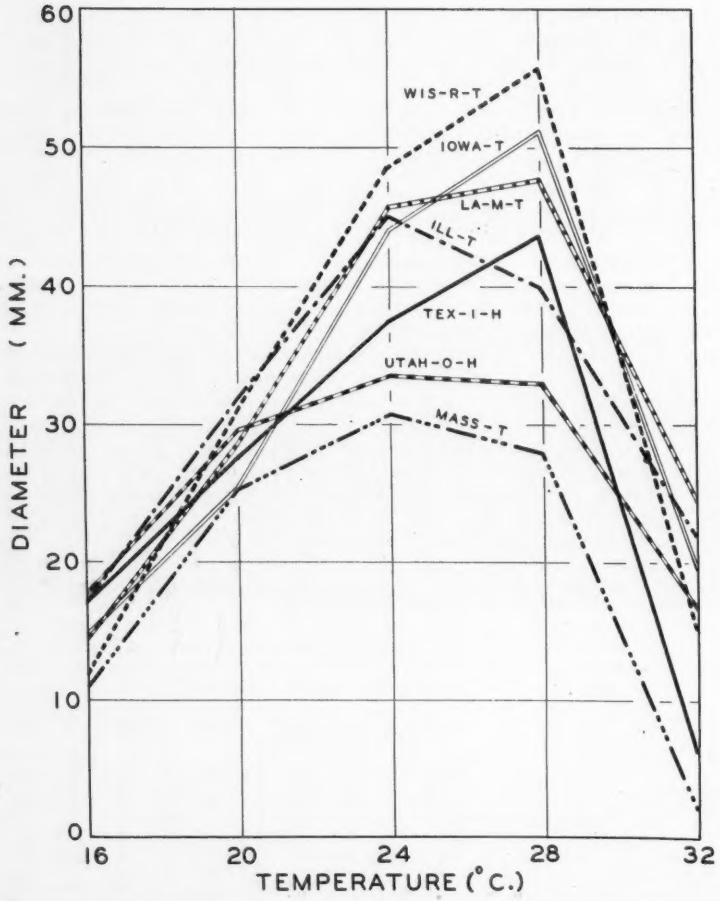

FIGURE 5.—Relation of temperature to diameter of colony of isolates Wis–R–T, Iowa–T, La–M–T, Ill–T, Tex–1–H, Utah–O–H, and Mass–T of *Pyrenochaeta terrestris* after 8 days.

to 25° were infected, whereas only 4 were infected at 30° and 15 at 20°. The best plant development occurred at 25°. Hansen concluded that the optimum for disease development was not far from 26°. The results of the present investigation (table 2) show that the index of isolate Utah–T increased with temperature up to 28°. In another experiment 4 varieties were grown in sand inoculated with isolates Tex–1–T, La–S–S, Iowa–S, and Wis–R–T at 16°, 20°, 24°, and 28°, respectively. The results are presented in table 6.

TABLE 6.—*Relation of 4 isolates of Pyrenochaeta terrestris and sand temperature to disease development* [1]

Variety	Isolate	Disease index at temperature indicated				Variety mean
		16° C.	20° C.	24° C.	28° C.	
Excel	Tex–1–T	79	93	99	100	78.5
	La–S–S	51	67	84	94	
	Iowa–S	38	73	93	97	
	Wis–R–T	35	70	90	94	
Texas Grano	Tex–1–T	84	99	100	100	74.0
	La–S–S	79	95	98	100	
	Iowa–S	25	41	76	94	
	Wis–R–T	23	30	59	83	
Mountain Danvers	Tex–1–T	76	97	99	100	67.8
	La–S–S	66	83	91	100	
	Iowa–S	23	35	50	82	
	Wis–R–T	21	28	57	78	
Yellow Globe Danvers	Tex–1–T	71	95	100	100	64.2
	La–S–S	65	84	90	98	
	Iowa–S	20	33	54	76	
	Wis–R–T	16	24	34	67	
Temperature mean		48.3	65.4	79.6	91.4	
Isolate mean (all varieties and all temperatures).	Tex–1–T	93.3				
	La–S–S	84.1				
	Iowa–S	56.9				
	Wis–R–T	50.6				

[1] Least significant difference:

	5-percent level	1-percent level
Varieties	2.7	3.5
Isolates	2.7	3.5
Temperatures	2.7	3.5
Within varieties, isolates, or temperatures	10.4	14.1

Isolate Tex–1–T was much more virulent than isolates Iowa–S and Wis–R–T and slightly more virulent than isolate La–S–S. The differences were more pronounced at 16° C. and usually became less so at each step increase in temperature. The most severe disease development was at 28°. Excel apparently was as susceptible to isolate Tex–1–T as Texas Grano, Mountain Danvers, and Yellow Globe Danvers. It was more susceptible than the last two varieties to isolates Wis–R–T and Iowa–S.

HYDROGEN-ION RELATIONS

Davis and Henderson (*1*) reported a pH range of 4.2 to 7.4 to be favorable to growth of the pink root fungus on potato-dextrose agar. In the present investigation growth was studied on a modified Hoagland's nutrient solution [7] containing 15 percent malt extract, 10 per-

[7] See footnote 6, p. 3

cent dextrose, and 20 percent agar. Into each plate after sterilization 20 cc. of the medium was measured and adjusted to pH 4, 6, or 8 with sodium hydroxide or hydrochloric acid. Five replicates of each were inoculated with isolates La–S–S, La–M–T, Utah–O–H, and Tex–1–T and incubated at 24° C. for 8 days. The measurements recorded in table 7 were nearly the same at all pH levels for three isolates; for the fourth isolate (Utah–O–H) there was a small decrease in growth from pH 8 to pH 4.

In a second experiment isolate La–M–T was grown at pH 2, 3, 4, 5, 6, 7, and 8, respectively. There was no difference in growth at pH 4 to 8; a decided retardation occurred at pH 3; and no growth occurred at pH 2.

TABLE 7.—*Growth of 4 isolates of Pyrenochaeta terrestris on malt-extract agar of 3 initial pH levels*

Isolate	Diameter of colony at initial pH level indicated		
	4	6	8
	Millimeters	*Millimeters*	*Millimeters*
La–M–T	54.6	54.4	55.2
La–S–S	46.8	46.2	44.8
Tex–1–T	53.6	55.6	55.4
Utah–O–H	48.8	52.2	56.0

Kreutzer (*4*) studied the nature of the influence of hydrogen-ion concentration on the manifestation of color in onion roots infected by the pink root fungus. He placed diseased roots in a liquid and changed the pH of the latter. The color changed from red or red purple at pH 8.5 to yellow brown at pH 4.5. In the present investigation four varieties of onion were grown in sand inoculated with four isolates and placed in a continuous-drip nutrition system (*6*) in which the nutrient was adjusted to pH 4, 6, and 8, respectively. The nutrient solutions at the two lower pH levels had changed very slightly when they left the sand culture. The pH 8 nutrient had shifted to about pH 7 when it left the pot. The disease indices are given in table 8. Isolate Utah–O–H was the least virulent and Tex–1–T the most virulent. Excel was consistently more resistant at all pH levels and to all isolates. There was no consistent difference in disease indices at the different pH levels. Root color varied with variety and isolate at each pH level. The darkest red roots were at pH 8; many brownish pink-roots were found at pH 4.

The heights of inoculated and uninoculated plants were recorded at the end of the experiment (table 9). There was no appreciable difference between plants at the three pH levels in the uninoculated sand. In the inoculated sand there was an inverse relation between height of plants and virulence of isolate, the greatest difference being between plants infected by isolate Utah–O–H and those by isolate Tex–1–T. Within a given isolate there was an inverse relation between disease index and plant height. The widest difference was that between Excel, the most resistant, and White Grano, the most susceptible variety. The difference was more pronounced with the three highly virulent isolates than with isolate Utah–O–H.

TABLE 8.—*Effect of initial pH on disease development on 4 varieties of onion inoculated with 4 isolates of Pyrenochaeta terrestris* [1]

Isolate	Variety	Disease index at initial pH level indicated			Mean for all pH levels	Isolate mean
		4	6	8		
Utah-O-H	Excel	5	15	10	10.0	20.8
	White Grano	20	35	35	30.0	
	White Sweet Spanish	15	25	25	21.7	
	Yellow Globe Danvers	15	25	25	21.7	
La-M-T	Excel	60	40	45	48.3	75.4
	White Grano	85	90	90	88.3	
	White Sweet Spanish	80	70	85	78.3	
	Yellow Globe Danvers	80	90	90	86.7	
La-S-S	Excel	30	30	40	33.3	70.4
	White Grano	80	85	90	85.0	
	White Sweet Spanish	80	80	90	83.3	
	Yellow Globe Danvers	75	80	85	80.0	
Tex-1-T	Excel	50	50	45	48.3	83.3
	White Grano	95	95	95	95.0	
	White Sweet Spanish	95	95	95	95.0	
	Yellow Globe Danvers	95	95	95	95.0	
Variety mean (all isolates and all pH levels).	Excel	35.0				
	White Grano	74.6				
	White Sweet Spanish	69.6				
	Yellow Globe Danvers	70.8				

[1] Least significant difference:

	5-percent level	*1-percent level*
Varieties	5.3	7.0
Isolates	5.3	7.0
Varieties × isolates	14.9	19.7

TABLE 9.—*Effect of initial pH on the height of seedlings of 4 varieties of onion inoculated with 4 isolates of Pyrenochaeta terrestris* [1]

Control or isolate	Variety	Height of seedings at—			Mean for all pH levels	Control or isolate mean
		pH 4	pH 6	pH 8		
		Inches	*Inches*	*Inches*	*Inches*	*Inches*
Control (uninoculated)	Excel	8.5	8.0	9.0	8.4	8.4
	White Grano	8.5	8.0	8.5	8.3	
	White Sweet Spanish	8.0	7.5	8.0	8.1	
	Yellow Globe Danvers	9.0	8.5	9.0	8.8	
Utah-O-H	Excel	7.0	7.0	7.5	7.1	6.9
	White Grano	6.5	7.0	7.0	6.7	
	White Sweet Spanish	6.5	6.5	6.5	6.5	
	Yellow Globe Danvers	7.5	7.0	7.5	7.3	
La-M-T	Excel	4.5	5.5	5.0	5.0	3.3
	White Grano	2.0	3.0	1.5	2.3	
	White Sweet Spanish	3.5	4.0	2.5	3.4	
	Yellow Globe Danvers	2.5	3.0	2.0	2.6	
La-S-S	Excel	5.0	5.5	5.5	5.3	3.8
	White Grano	2.0	3.5	3.0	2.8	
	White Sweet Spanish	3.0	3.5	3.0	3.3	
	Yellow Globe Danvers	4.0	4.0	3.0	3.8	
Tex-1-T	Excel	4.0	5.0	4.5	4.5	3.2
	White Grano	2.0	3.0	3.0	2.8	
	White Sweet Spanish	3.0	3.5	3.0	3.1	
	Yellow Globe Danvers	2.5	2.5	2.5	2.5	
Variety mean (all isolates and all pH levels).	Excel	5.5				
	White Grano	3.7				
	White Sweet Spanish	4.1				
	Yellow Globe Danvers	4.1				

[1] Least significant difference:

	5-percent level	*1-percent level*
Varieties	0.4	0.5
Isolates	.4	.6

HOST RESISTANCE

Taubenhaus and Mally (*8*) reported that in Texas the Bermuda varieties of onion were moderately resistant to pink root, whereas other varieties tested, including Sweet Spanish, were very susceptible. Porter and Jones (*5*) found that in California Australian Brown and Yellow Globe Danvers were susceptible and Sweet Spanish only moderately so. In the present investigation results from the experiments already reported showed a difference in degree of pink root development in varieties grown for 28 days on heavily infested sand at temperatures very favorable for the causal fungus. In most instances Yellow Bermuda and Excel showed lower indices than other varieties tested. This is in accord with the reputed behavior of Bermuda varieties in infested soil in the Rio Grande Valley and elsewhere. The experiments also showed that the expression of resistance was suppressed as the temperature rose to 28° C. and in proportion to the virulence and concentration of the pathogen. Under optimum conditions, then, Yellow Bermuda eventually showed a disease index of 100.

The optimum conditions for severe seedling tests having been worked out, various onion varieties were tested for susceptibility to a large number of isolates. The data from the first series are given in table 10. The disease indices of isolates Mass–T, Col–FC1–T, Wis–R–T, and Wis–M–T averaged highly significantly lower than those of the other isolates. Yellow Bermuda–1 had a highly significantly lower index than any other variety tested, and this difference was usually significant when varieties were compared within each individual isolate. Excel–1 was the next in resistance, but its index was not so consistently low as that of Yellow Bermuda–1.

TABLE 10.—*Reaction of onion varieties to isolates of Pyrenochaeta terrestris, series 1* [1]

Variety	Disease index of isolate indicated										Variety mean
	Cal–D–T	La–S	Ill–T	Tex–1–S	Col–FC1–T	Iowa–S	Utah–T	Mass–T	Wis–M–T	Wis–R–T	
Nebuka	96	81	98	55	38	95	64	44	77	62	71.0
Yellow Bermuda–1	71	72	83	62	29	80	63	29	54	37	58.0
Excel–1	96	75	94	61	33	96	70	37	56	74	69.2
Brigham Yellow Globe–1	96	86	81	86	55	78	84	43	56	54	71.9
Brigham Yellow Globe–2	95	88	86	86	57	81	91	44	66	48	74.2
Red Wethersfield–1	95	88	83	83	50	82	89	41	70	48	72.9
Yellow Globe Danvers–1	98	87	86	82	53	83	89	45	64	54	74.1
Yellow Globe Danvers–2	98	89	87	86	58	87	89	50	72	52	76.8
Early Yellow Globe–1	99	90	85	90	62	85	96	46	67	56	77.6
Ebenezer–1	96	94	83	87	56	86	89	43	67	55	75.6
Sweet Spanish–1	97	88	89	79	46	88	89	58	76	58	76.8
White Sweet Spanish–1	95	92	85	80	52	82	89	50	74	57	75.6
Australian Brown–1	98	92	94	86	52	89	93	44	74	55	77.7
Sutton's A1	98	95	91	88	56	92	89	55	69	60	79.3
Ailsa Craig	100	100	100	97	69	99	96	75	80	76	89.2
Autumn Queen	98	95	100	88	70	96	92	73	89	84	88.5
Isolate mean	95.4	88.3	89.1	81.0	52.3	87.4	85.8	48.6	69.4	58.1	

[1] Least significant difference:

	5-percent level	*1-percent level*
Varieties	3.4	4.5
Isolates	2.7	3.6
Varieties × isolates	15.2	20.1
Within varieties or isolates	10.7	14.2

The data from the second series are given in table 11. The disease indices of isolates Cal–D–T, La–S–S, Ill–T, and Col–FC2–T averaged significantly higher than those of the other isolates. Beltsville Bunching (an amphidiploid) was the most resistant variety, followed by Excel–2 and California Hybrid Red. Excel–2 was grown from a fresh lot of seed, whereas Excel–1 was grown from seed of the previous year. Thus the slightly higher disease index of Excel–1 may have been due partly to less vigorous growth after germination. All the other varieties tested were uniformly susceptible.

TABLE 11.—*Reaction of onion varieties to isolates of Pyrenochaeta terrestris, series 2* [1]

Variety	Disease index of isolate indicated										Variety mean
	Cal–D–T	La–S–S	Ill–T	Tex–1–S	Col–FC2–T	Iowa–1–T	Utah–T	Mass–T	Wis–M–T	Wis–R–T	
Beltsville Bunching	99	100	94	35	99	35	41	62	87	77	72.9
Excel–1	97	100	97	69	99	76	65	73	94	97	86.7
Excel–2	98	98	99	54	98	73	57	70	97	91	83.5
Brigham Yellow Globe–1	100	100	96	81	100	76	94	77	93	89	90.6
Brigham Yellow Globe–3	100	100	94	92	100	82	94	84	92	91	92.9
Red Wethersfield–2	100	100	96	78	100	77	90	79	92	91	90.3
Yellow Globe Danvers–1	100	100	99	82	100	76	94	77	91	87	90.6
Yellow Globe Danvers–2	100	100	99	81	100	83	96	81	95	91	92.6
Early Yellow Globe–1	100	100	98	91	100	72	97	75	91	89	91.3
Ebenezer–1	100	100	97	80	100	70	91	71	90	89	88.8
Sweet Spanish–1	100	100	98	73	100	80	85	75	98	95	90.4
White Sweet Spanish–1	100	100	99	79	100	77	94	77	96	92	91.4
Australian Brown–1	100	100	100	73	100	80	90	85	95	98	92.1
California Hybrid Red	100	100	95	82	100	61	93	67	72	78	84.8
Texas Grano	100	100	98	82	100	69	96	75	96	87	90.3
Isolate mean	99.6	99.9	97.3	75.5	99.7	72.5	85.1	75.2	91.9	89.5	------

[1] Least significant difference:

	5-percent level	*1-percent level*
Varieties	2.4	3.2
Isolates	1.9	2.5
Varieties × isolates	10.7	14.1
Within varieties or isolates	7.6	10.0

The data from the third series are presented in table 12. Again the disease indices of isolates Cal–D–T, La–S–S, and Col–FC2–T were much higher than those of the other isolates. Yellow Bermuda–2 and White Sweet Spanish–2 were significantly more resistant than the other varieties. Crystal Grano was significantly more susceptible than the other varieties.

A comparison of disease indices of sand- and soil-culture series is given in table 13. The indices for the sand-culture series represent an average of all varieties inoculated with a given isolate. It can be seen that there was a general tendency for the indices of the soil series to be lower than those of the sand series. There was also more variation in the soil-culture series. Isolates Cal–D–T, La–S–S, and Col–FC2–T gave consistently high indices in both soil and sand culture. The reaction of the solutions siphoned from the pans was approximately pH 7.3. There was no significant difference between the reaction of solutions from pans inoculated with the various isolates.

TABLE 12.—*Reaction of onion varieties to isolates of Pyrenochaeta terrestris, series 3* [1] [2]

Variety	Disease index of isolate indicated										Variety mean [1]
	Cal-D-T [1]	La-S-S [1]	Ill-T [1]	Tex-2-S	Col-FC2-T [1]	Iowa-S	Utah-T	Mass-T	Wis-M-T	Wis-R-T	
Yellow Bermuda-2	95	90	63	30	100	80	40	60	73	65	58.0
White Sweet Spanish-2	94	93	70	50	100	80	60	65	65	48	61.3
White Sweet Spanish-3	96	96	93	65	100	85	65	95	99	85	82.3
Sweet Spanish-2	99	100	85	55	100	89	75	90	88	80	79.5
California Early Red	100	95	95	75	100	73	93	70	75	65	75.2
Crystal Wax	98	93	83	55	100	86	65	83	73	60	70.3
Australian Brown-2	100	90	80	60	100	80	90	60	70	60	70.0
Lord Howe Island	99	100	83	55	100	92	75	70	65	70	71.2
Southport Red Globe	98	99	75	55	100	88	85	68	80	63	73.2
Yellow Globe Danvers-3	100	100	80	60	100	90	85	75	63	75	74.7
Ebenezer-2	100	100	83	50	100	91	90	78	75	65	74.8
White Babosa	100	100	90	55	100	95	80	80	85	65	76.7
Early Yellow Globe-2	100	100	83	55	100	94	98	88	80	70	80.8
White Grano	100	100	83	70	100	92	95	80	83	73	82.2
White Portugal	100	98	90	75	100	90	90	95	95	80	87.5
Crystal Grano	100	100	100	80	100	94	100	99	99	93	94.2
Isolate mean	98.7	97.1	83.5	59.1	100.0	87.4	80.4	78.5	79.3	69.8	

[1] Disease-index values for isolates Cal-D-T, La-S-S, Ill-T, and Col-FC2-T not used in the determination of variety means and in the statistical analysis.

[2] Least significant difference:

	5-percent level	*1-percent level*
Varieties	5.9	7.8
Isolates	3.6	4.8
Varieties × isolates	20.5	26.9
Within varieties or isolates	14.5	19.1

TABLE 13.—*Disease indices in unsterilized soil and in sand inoculated with various isolates of Pyrenochaeta terrestris*

Isolate	Disease index in series and substrate indicated					
	Series 1		Series 2		Series 3	
	Sand	Soil	Sand	Soil	Sand	Soil
Cal-D-T	95	90	99	88	99	78
Utah-T	86	10	84	25	80	0
Ill-T	89	70	96	55	84	5
Wis-M-T	69	50	90	80	79	10
Wis-R-T	58	65	88	60	70	40
Mass-T	49	60	75	30	80	10
La-S	88	65				
La-S-S			100	100	97	80
Col-FC1-T	52	90				
Col-FC2-T			100	100	100	95
Tex-1-S	81	25	75	60		
Tex-2-S					59	1
Iowa-S	87	35			87	5
Iowa-1-T			72	50		

DISCUSSION

One of the objects of the present investigation was to develop a standardized technique whereby large numbers of individual onion plants could be tested for their resistance or susceptibility to *Pyrenochaeta terrestris*. The experimental results show that highly uniform and reproducible results can be obtained by using inoculated white

quartz sand as a substrate, in which the complex introduced by soil flora and other soil factors is removed. By the use of Yellow Bermuda, the most resistant variety in field tests, as a standard, it was demonstrated that an index of resistance could be obtained on seedlings in about 28 days. The expression of resistance, however, was influenced by a number of environmental factors; in fact conditions were created in which Yellow Bermuda was completely killed. The severity of disease, as measured by the index described, increased up to a certain point with increase in concentration of inoculum and in the temperature maintained in the sand. Isolates from various parts of the United States and those from within a given area varied in degree of virulence. In general, isolates carried in culture by mass transfer maintained about the same degree of virulence over a period of 18 months; there was little indication that they differed greatly in their selective pathogenicity to the varieties studied.

Twenty-three standard varieties were tested by mixing 100 cc. of standardized inoculum with 10 kg. of sand and growing seedlings therein for 28 days. All seedlings, even those of the Yellow Bermuda–2, were killed promptly by the most virulent isolate (Col–FC2–T, table 12). When isolates of intermediate virulence (Utah–T and Ill–T, table 12) were used a large percentage of the Yellow Bermuda–2 plants survived, but all plants of Crystal Grano, the most susceptible variety, succumbed. Under the controlled conditions just described an isolate of intermediate virulence was used satisfactorily to screen several hundred breeding progenies from which the most resistant segregates had to be selected. Obviously an extremely virulent pathogen might have been selected as a standard, and the rate of disease development might have been retarded by reducing the concentration of the inoculum or the sand temperature or both to provide conditions under which a certain percentage of the Yellow Bermuda standard would survive. The point emphasized here is that by using the results of this investigation it was possible to set up a standard test in which environment and virulence of pathogen were controlled to the point at which screening tests from month to month and year to year were reasonably comparable; such a test could not be developed in the field.

SUMMARY

A large number of isolates of the pink root fungus (*Pyrenochaeta terrestris*) from various parts of the United States were assembled and studied.

The optimum temperature for growth of the various isolates was 24° or 28° C. There were marked differences in rate of growth between isolates, especially at 28°. They grew well at all hydrogen-ion levels tested within the range pH 4 to 8. The isolates, even those from the same section, were found to differ widely in pathogenicity.

Maximum pink root development in sand culture occurred at 28° C. Disease development in sand cultures to which the nutrient solutions were added by the use of a drip system was not affected by a change in pH within the range pH 4 to 8.

A pan, sand-culture method was developed. By the use of standardized inoculation procedure and controlled environment uniform infection of large numbers of seedlings could be obtained. By this method a survey was made of the resistance of 23 commercial varieties of onion to various isolates of *Pyrenochaeta terrestris* from widely separated areas. Yellow Bermuda and Beltsville Bunching were the most resistant varieties tested. Excel, a selection from Yellow Bermuda, approached but did not equal Yellow Bermuda in resistance.

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BACTERIOLOGICAL CHANGES DURING THE FERMENTATION OF STEAMED POTATOES FOR SILAGE[1]

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INTRODUCTION

During the summer of 1944 the War Food Administration sponsored experimental work at a number of State agricultural experiment stations on the utilization of surplus potatoes (*Solanum tuberosum* L.) for stock feed. The use of the surplus in the form of silage for livestock was stressed, and general recommendations as to the procedures to be followed were given.[3] It was stated that the best method was that of steaming and ensiling in pits.

The experimental studies at the North Carolina station were based on three carloads of potatoes and involved the cooperative efforts of several departments (Agricultural Engineering, Animal Industry, and Horticulture) as well as the Bureau of Agricultural and Industrial Chemistry of the United States Department of Agriculture. The joint work was planned to cover two general phases, namely, bacteriological and chemical changes during the fermentation and feeding trials on the ensiled material.

This report deals with the principal bacteriological changes occurring during the fermentation of the steamed potatoes. Such studies are of considerable interest in view of the nature of the preparation of this type of silage which calls for steaming the potatoes until cooked and promptly ensiling (while hot) relatively large tonnages in pits. Such a procedure would naturally introduce the question of the effect of prolonged high temperature upon the micro-organisms associated with normal silage fermentations, especially the lactic acid bacteria (of the *Lactobacillus* genus) and other non-heat-resistant types, such as the yeasts and coliforms. While the preparation and use of steamed potato silage has been the subject of investigation for many years, principally abroad, the bacteriological changes have not been clearly indicated.

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[2] The writers wish to express their thanks to the War Food Administration for furnishing the potatoes used in the experiments, and to C. Hillman Moody, administrator of the War Food Administration for North Carolina, for his cooperation and interest in the work.

[3] BRANDT, K., and KRAEMER, J. RECOMMENDATIONS FOR UTILIZATION OF SURPLUS POTATOES BY STEAMING AND ENSILING. 10 pp., 1944. [Mimeographed.]

PROCEDURE

Forty-five tons of U. S. No. 1, size B, potatoes were available for the work. The potatoes were shipped in carload lots from the eastern part of North Carolina and arrived in good condition. No attempt was made to remove the few rotten potatoes occasionally found. Thirty tons were used for the steamed-silage study and 15 tons were ensiled raw. Only the work on the steamed silage is discussed in this paper.

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EXPERIMENTAL SILO

The experimental trench silo used was one of two constructed at the Central Experiment Station farm near the college. These silos had approximately the following dimensions: 6 feet deep; 8 feet wide at the bottom, 11½ feet wide at the top; and 25 feet long. They had concrete floors and plastered concrete walls 3 to 4 inches thick, reinforced with wire fencing. The ends were closed by the use of 2- × 6-inch planks, supported by 4- × 4-inch posts. Both silos were provided with covers and were constructed in a hillside to provide natural drainage (fig. 1, *A*).

STEAMING OPERATION

The potatoes were steamed on July 10 and 11 at the college, two steel-bodied dump trucks being used. These trucks had a capacity of from 1½ to 2 tons of raw potatoes. On the bottom of each truck body was fastened a steam rake consisting of four parallel lengths of perforated ½-inch pipe connected to a horizontal cross member to which a vertical inlet was attached. The rake was connected to the steam line by means of a steam hose and pipe. The steam pressure was 120 pounds per square inch.

The potatoes were dumped into the trucks directly from the railroad cars, covered with heavy tarpaulins, and steamed until cooked throughout (fig. 1, *B* and *D*). This required from ¾ to 1 hour with the equipment used. The potatoes were kept covered and taken immediately to the trench silo (about a 10-minute trip) and dumped (fig. 1, *C*). In this manner, the 30 tons of potatoes were handled in about 12 working hours, using two trucks and two sources of steam.

FILLING THE SILO

The potatoes were dumped into the silo at three locations along one side. When all the steamed potatoes were in the trench, they were leveled off and covered with lapped strips of heavy tar-paper roofing (fig. 1, *E*). The mass was packed by carefully tamping the surface of the paper. A scant 4-inch layer of soil was then added for weighting and sealing. The 30 tons of steamed potatoes filled the trench to a depth of 3½ to 4 feet. About 30 hours after the first load had been steamed and dumped and about 6 hours after the last load had been put in the silo the temperature at the approximate center of the mass was in the range of 160°–164° F. Subsequent temperature readings were taken at two

FIGURE 1.—Ensiling steamed potatoes. *A*, Experimental trench silo with three-piece cover, one of which (background) has been removed preparatory to dumping in the potatoes. *B*, Steaming the covered potatoes in a metal-bodied dump truck. *C*, Dumping the steamed potatoes in the front (north) section of the trench. *D*, Steamed potatoes in the trench before leveling and covering; consistency of potatoes at this stage shown in area struck by the shovel. *E*, The filled trench at the time of the leveling and covering operation; the dial thermometer (right foreground) read approximately 160° F. at the conclusion of filling and covering. *F*, Sampling: A core of the top layer of surface potatoes removed with a petri-dish can (left) prior to taking the bacteriological sample with the smaller pipette can (right). *G*, Part of the end (south) section of the ensiled potatoes opened after 8 months' storage; the potatoes form a tightly packed mass beneath the light-colored soil layer. *H*, Close-up of ensiled potatoes after cutting through a small area near the top; cavities represent areas where individual potatoes fell away.

locations at about mid-depth in the mass. A copper-constantan thermocouple was placed at one location and the bulb of a vapor-actuated dial thermometer at the other.

SAMPLING TECHNIQUE

Samples were taken for bacteriological and chemical examination in the following manner: First, the soil was carefully removed from an area of tar-paper roofing (about 18 inches square) with a garden hoe, and the surface was brushed clean with a whisk broom. Then a U-shaped cut (8–10 inches on a side) was made in the paper and the flap was turned back. A sterile, copper petri-dish can (4½ inches in diameter and 8¼ inches long) was inverted and pushed vertically into the mass, and an 8-inch core of the top layer of potatoes was thus removed and discarded (fig. 1, *F*). The actual sample was obtained by removing a second core of potatoes with a sterile, copper pipette can (2 inches in diameter and 12 inches long) starting from the center of the surface left by the first cut. This gave about a 1½-pound sample from the central layer about 1 to 2 feet beneath the surface. The temperature of each sample area was taken by means of a maximum-indicating thermometer inserted into the wall of the cut at the time the sample was removed. After the sample was taken, the potatoes from the discarded core were tamped back into the hole. The tar-paper flap was then folded back in place and a new square of tar-paper larger than the opening was placed over the surface and covered with soil, after which the location was recorded. The sampling was restricted to about one-half of the total silo (fig. 2). This section was found to be the hottest

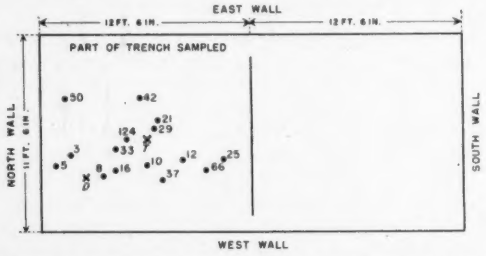


FIGURE 2.—Diagram representing top surface of the trench silo and indicating the comparative location of points at which core samples were taken for analysis. *D* marks location of the dial-type thermometer and *T*, the location of the thermocouple; the numbers indicate the age of the fermentation in days at each sampling.

after the filling operation was complete. Difference in temperature in opposite end portions of the ensiled mass was to be expected because of the method of filling the silo. This resulted in the space at the lower end of the silo being filled with cooler material, particularly after leveling.

MEDIA AND METHODS

After the sample was collected, it was taken promptly to the laboratory for analysis. Fifty grams of the potato mass was finely cut with a sterile spatula into a glass-top mason jar containing 450 grams of

sterile saline. The 1:10 mixture was shaken 100 times by hand prior to making suitable dilutions for use with solid and liquid media. Duplicate tests on 50-gram subsamples were run at each sampling interval. The remainder of the potato sample was reserved for chemical analysis by sealing it in glass jars and storing in a freezing cabinet. The major chemical changes occuring during the fermentation are not given in this report, but the titratable acidity and pH values, based on examination of the 1:10 dilution used for bacteriological analysis, are included.

Bacteriological examination of the silage was made for various mesophilic and thermophilic groups of micro-organisms. These are listed in table 1 together with the liquid or solid medium used and reference to the general procedure followed. For the most part, the procedures for the mesophiles have been successfully used by the authors for a number of years in connection with brined vegetable and sweetpotato-vine-silage fermentations (*5, 6, 7, 8, 9, 10*).[4] The methods of examination for the thermophilic groups are essentially those developed in the laboratories of the National Canners Association (*3, 4*). It should be mentioned that in examinations for viable aerobic (*13*) and anaerobic sporeforms (*3*) by use of the media indicated, a boiled sample (2 minutes) of the 1:10 silage dilution was used which had been previously neutralized with sterile calcium carbonate. The same was true for the H_2S-producing thermophilic anaerobes. The counts for facultative thermophilic anaerobes are based on colonies resulting both from viable cells and spores from unboiled samples.

TABLE 1.—*Estimates of micro-organisms on potatoes (U. S. No. 1, B size) before and after steaming for silage*

Microbial group	Culture medium used and procedure reference	Count per milliliter of wash water [1]	
		Raw potatoes	Steamed potatoes [2]
Mesophiles (35° C. incubation):			
Total count	Nutritive caseinate agar+BCP [3]; plates (*10, 7*).	86,000,000	200
Lactic acid bacteria	Nutritive caseinate agar+BCP; plates (*10, 7*).	[4] 0	0
Coliform group	Brilliant green agar; plates (*8, 9, 6*)	25,000,000	0
Yeasts	Acidified dextrose agar; plates (*5, 10*)	150,000	0
Molds	Acidified dextrose agar; plates (*5, 10*)	160,000	0
Aerobic spore count	Nutritive caseinate agar+BCP; plates (*13*).	22,000	100
Anaerobic spore count [5]	Liver broth+particles; tubes (*3*)	100	10
Thermophiles (55° incubation):			
Facultative anaerobes	Dextrose tryptone agar+BCP; plates (*3*).	3,600	40
Non-H_2S-producing anaerobes	Liver broth+particles; tubes (*3*)	10	10
H_2S-producing anaerobes	Sulfite agar; tubes (*4*)	10	10

[1] 450 grams of whole potatoes plus 450 grams of sterile saline shaken 100 times.
[2] Taken from a 1- to 1½-ton lot steamed 45 minutes to 1 hour in a steel-bodied dump truck covered with a heavy tarpaulin; steam pressure at the source was 120 pounds per square inch.
[3] Bromcresol purple; 0.04 gram per liter.
[4] Not detected because of predominance of and overgrowth by alkaline and coliform types.
[5] Includes putrefactive types as well as true butyric types of gas-forming, obligate anaerobes.

[4] Italic numbers in parentheses refer to Literature Cited, p. 31.

MICROSCOPIC COUNTS

At each sampling interval, 0.01-milliliter portions of the original 1:10 silage dilutions were placed in sequence on duplicate slides for microscopic counting. The smears were prepared and counted according to the method of Wang (*14*), a modification of the Breed (*2*) technique. One set of slides was stained with the Kopeloff and Cohen (*11*) modification of the Gram stain, and the number of individual Gram-positive cells in 100 fields per smear was determined. For the other set, the Schaeffer and Fulton modification (*12*) of the Wirtz (*15*) spore stain was used, and the number of individual spores in 100 fields per smear was noted. The microscopic counts for both cells and spores are reported in terms of millions per gram of silage.

TITRATABLE ACIDITY AND pH

As mentioned previously, titratable acidity and pH determinations were made on the original 1:10 dilution of the silage used for bacteriological purposes The pH determinations were made with the glass electrode. Titratable acidity was run on 10-milliliter aliquots of the 1:10 dilution (after allowing it to stand 1 to 2 hours) by titrating with 0.111 N NaOH using phenolphthalein as the indicator. The values were calculated in terms of grams lactic acid per 100 grams of potato silage. These determinations were made on the 1:10 dilution for the purpose of having available, at the time of plating, a reasonably clear picture of the progress of acid production during the fermentation.

RESULTS

EFFECT OF STEAMING ON SURFACE ORGANISMS

At the time the steaming operation was going on, an attempt was made to obtain an estimate of the relative number of different groups of organisms occurring on the surface of the uncut potatoes both before and after steaming. The results are given in table 1. It is evident that the steaming procedure greatly reduced the large number of surface organisms. The relatively few that survived were the heat-resistant, spore-forming types. As far as could be determined, the usual types of organisms associated with normal silage fermentations (i. e., lactic acid bacteria, yeasts, and coliforms) did not survive the heating. Although the initial count of the facultative anaerobic thermophiles was small in comparison with the total number of organisms present on the raw potatoes, the proportion of the thermophiles surviving the steaming operation was much greater. This is of considerable importance in view of their role in the subsequent fermentation of the ensiled potatoes.

TEMPERATURE CHANGES DURING FERMENTATION OF SILAGE

After the initial steaming operation, the surviving micro-organisms on the potatoes were subjected to further prolonged exposure to rather high temperatures during the time required for the hot mass to cool in the silo. Progressive temperature changes based on readings made repeatedly at two locations (fig. 2) in the silo are shown

in table 2. The location of each individual area sampled and the temperature of that particular area at the time of sampling are also presented. In general, the temperature of the mass 2 days after ensiling was in the range of 160° F. Furthermore, it remained above 120° for about the first 12 days and above 100° for the first 3 weeks. The dial thermometer location shows higher temperature readings during the first few days and lower readings after 12 days than the thermocouple location. This is accounted for by the fact that the thermometer was located where the steamed potatoes were dumped, which was the hottest area. However, it was also closer to the front and side walls, and therefore cooled faster than the more central location occupied by the thermocouple.

TABLE 2.—*Temperature changes during the fermentation of steamed potato silage*

Date (1944)	Age	Distance of area sampled from—		Temperature record			
		North wall	West wall	Sample area	Dial thermometer [1]	Thermocouple [2]	Atmosphere
	Days	*Inches*	*Inches*	° F.	° F.	° F.	° F.
July 11, 8 p. m.	1					160	82
12, 2:30 p. m.	2				164	158	91
13, 8 a. m.	3	24	52	162	159	152	81
15, 2:30 p. m.	5	12	46	136	145	140	86
18, 2:30 p. m.	8	46	38	132	131	132	77
20, 1:30 p. m.	10	76	46	126	124	127	90
22, 2:30 p. m.	12	102	51	122	118	122	82
26, 1:30 p. m.	16	52	42	108	108	114	96
31, 2 p. m.	21	82	76	104	101	108	87
Aug. 4, 1:30 p. m.	25	130	51	100	95	103	94
8, 1:30 p. m.	29	80	72	100	92	100	76
12, 10 a. m.	33	54	58	96	90	97	85
16, 10 a. m.	37	87	36	92	89	95	84
21, 10 a. m.	42	70	92	90	85	92	81
29, 11 a. m.	50	16	92	89	85	88	78
Sept. 14, 11 a. m.	66	118	42	84	82	84	80
Nov. 11, 2 p. m.	124	60	64		65	67	64

[1] Located 33 inches from the north wall and 33 inches from the west wall.
[2] Located 76 inches from the north wall and 66 inches from the west wall.

POPULATION OF MICRO-ORGANISMS DURING FERMENTATION OF SILAGE

The results of the bacteriological examination for the presence of various types of organisms during the silage fermentation are shown in table 3. Changes in acidity and pH of the ensiled potatoes as well as the approximate temperature of the core sample at each sampling interval are also given. The data indicate that the coliforms, yeasts, and lactic acid bacteria, groups usually associated with normal silage fermentation, did not contribute to the fermentation of this type of silage. This would seem reasonable as these groups are not heat-resistant and there was no evidence that they survived the steaming operation. Even if some few individual cells from these groups had survived the cooking, and some had been added during the filling operation, it is doubtful whether they would have withstood the elevated temperatures of the silage during the first 12 days.

TABLE 3.—*Populations of micro-organisms occurring during the fermentation of steamed potatoes for silage*

Sample date, 1944	Fermentation age	Temperature of sample	Thermophiles (55° C.), count per gram				Mesophiles (35° C.), count per gram					Microscopic count—individual cell and spore count per gram		pH	Titratable acidity as lactic acid
			Facultative anaerobes		Anaerobes		Total count	Acid-forming colonies	Coliforms, yeasts, lactic acid bacteria, and molds [1]	Spore forms		Gram+ cells	Spores		
			Total count	Acid-forming colonies	Non-H_2S-producing	H_2S-producing				Aerobes	Anaerobes [1]				
	Days	*°F.*	*Millions*	*Millions*			*Millions*	*Millions*		*Hundreds*	*Hundreds*	*Millions*	*Millions*		*Percent*
July 13	3	162	0.093	0.093	<10		0.001	<0.001	0	0.95	0	2.8	1.8	5.50	0.40
15	5	136	.350	[2] 0	<10	<10	.025	<.001	0	3.5	0	6.6	4.8	5.30	.50
18	8	132	1.9	1.9	1,000	<10	.041	<.001	0	2.0	0	4.0	6.6	4.70	.70
20	10	126	2.0	2.0	<10		2.13	2.13	0	<.1	0	15.2	9.0	4.78	.58
22	12	122	[3] 5.1	5.1	100	<10	2.5	2.5	0	13.0	1	11.6	25.2	4.90	.48
26	16	108	2.8	2.8	100		22.5	22.5	0	4.5	10	19.2	6.4	4.20	.87
31	21	104	7.6	7.6	1,000		13.5	12.5	0	2.3	1	32.0	70.8	4.11	1.18
Aug. 4	25	100	1.9	1.9	100	<10	10.3	10.2	0	.6	0	14.2		4.60	.60
8	29	100	11.5	11.5	<10		6.7	6.7	[4]	18.0	0	33.8	45.6	4.00	1.35
12	33	96	[5] .11	.01	<10		10.9	10.9	0	1.1	0	182.4	6.8	4.17	1.10
16	37	92	.25	.16	<10		1.4	1.4	0	13.5	1	50.8	37.6	4.05	1.15
21	42	90	15.0	11.5	100		9.0	[2] 0	0	11.5	0	52.0	101.2	4.03	1.83
29	50	89	4.1	4.1	<10	<10	4.6	4.5	0	505.0	0	210.0	14.0	3.96	1.36
Sept. 14	66	84	2.7	2.7	<10		1.6	1.1	0	750.0	0	64.6	23.4	3.90	1.50
Nov. 11	124	67	1.0	.25	<10	<10	.27	>.01	0	19.0	1	210.4	63.8	3.95	3.40

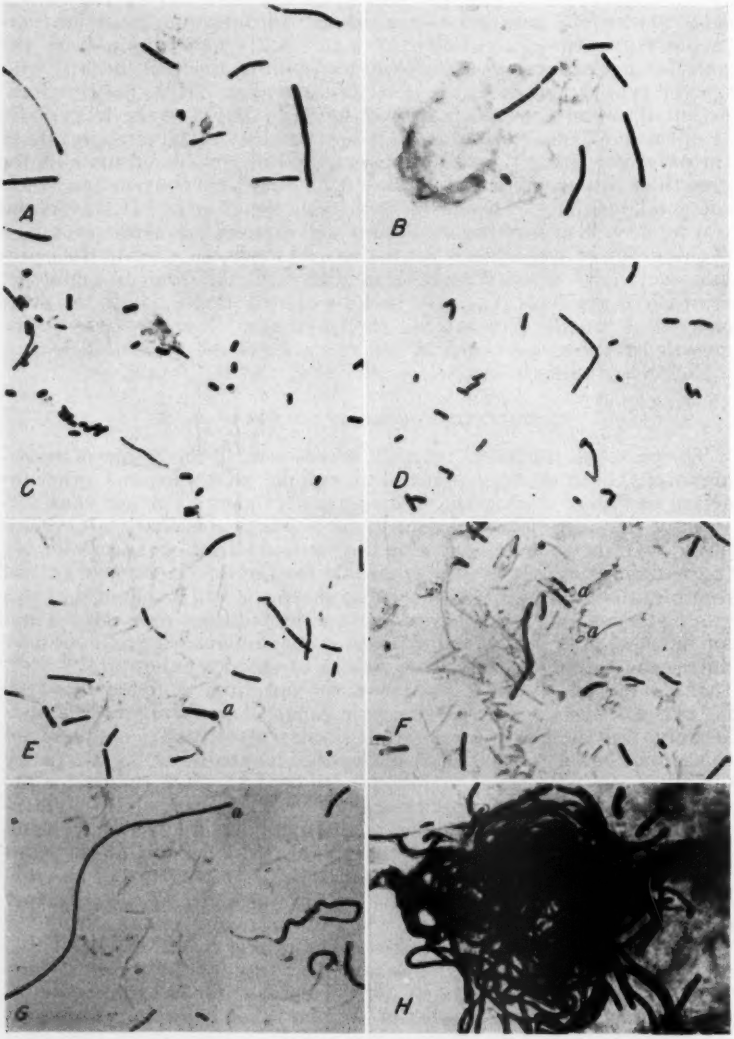
[1] Zeros in column indicate less than 10 per gram.
[2] Not detected because of predominance of and overgrowth by alkaline types.
[3] Spore count by plating boiled sample was 1.3 millions per gram.
[4] At this interval a count of 750 yeasts and 2,200 molds per gram was obtained, indicating probable contamination from the surface layer during sampling.
[5] Total count at 55° C. was 24 million per gram, using nutritive caseinate agar.

Examination of the silage with respect to certain of the anaerobic spoilage groups of bacteria, namely, the non-H_2S- and the H_2S-producing thermophiles, showed the former group to be present only in relatively small numbers on several occasions. The latter group appeared to be absent or was present to the extent of less than 10 per gram at the six times when examinations were made. Active growth by these two groups, if present, would have been expected during the 12-day interval when the potatoes were at or near the optimum range for thermophilic growth. Essentially the same results were obtained for the putrefactive anaerobes (mesophiles) including the true butyric and butylic types of gas-forming, obligate anaerobes. Here positive indication of the presence of spores was noted in only 5 of the 15 periodic samples and then only in relatively low numbers. The temperature of the potatoes during the first 12 days would be considered too high for growth of this group of anaerobes. After that time the reaction of the silage (about pH 4.5) would be unsuitable for growth. However, the test for growth of spore forms from boiled samples for certain members of this group of organisms is not necessarily a reliable index to their previous activity. Spore formation may be negligible prior to inhibition or death of the vegetative cells because of acid production in the presence of a readily fermentable carbohydrate. Nevertheless, active growth by these organisms in the silage would be associated with a malodorous fermentation. This condition was not found.

PREDOMINATING GROUPS OF MICRO-ORGANISMS

The previous discussion has dealt with several of the groups of micro-organisms, both thermophilic and mesophilic, that appeared either inactive or absent during the fermentation proper. Further consideration of the bacteriological data in table 3 indicates that the thermophilic, facultative anaerobes were the predominating organisms during the fermentation and were responsible for the developed acidity and resultant decrease in pH of the ensiled mass. It will be noted that this group started activity rather soon after the potatoes were ensiled and for the most part showed populations in the millions per gram not only during the period when the mass was at elevated temperatures (120°–160° F.) but also when it was below the optimum temperature range for obligate thermophiles. The wide range of temperature tolerance accounts for the presence of rather similar plate counts obtained for this group listed in table 3 as mesophiles incubated at 35° C. (using nutritive caseinate agar). However, the 55° C. plate counts for the first three sampling periods, during the very early phase of the fermentation, indicate principally obligate thermophiles involved since comparatively few colonies were noted on the 35° C. plates at the same period. The facultative relationship toward temperature of the predominting types, after active fermentation was under way, was further demonstrated by the fact that, when the principal colonies from the 55° C. routine plates were transferred into liver broth and incubated at 35° C., growth occurred. The same was true for those picked from the 35° plates and incubated at 55°. Hence, so far as could be determined from the limited number of samples taken from the trench during the active acid fermentation, the predominating groups that grew out at either incubation temperature (55° or 35°) were essentially the

same. There was one exception to this. In certain instances during the routine examinations, one specific colony type grew well at 35° on nutritive caseinate agar, but very poorly, if at all, at 55° on dextrose tryptone agar. However, this was found to be a problem of nutritive requirements concerning the two media used rather than temperature, as will be discussed later.



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The routine plate counts during the fermentation period further showed that the major portion of the total count in most instances was composed of acid-forming colonies of the thermophilic, facultative anaerobic group. This was true for the plates incubated at either 35° or 55° C. These organisms brought about an acid fermentation that resulted in acidification of the ensiled mass and consequent preservation. The general course of acid development is shown by the lowering of the pH from about 5.5 at 3 days to about 3.95 at 50 days and thereafter until the conclusion of the sampling. During this period the titratable acidity increased from about 0.4 percent (calculated as lactic acid) to about 3.0 percent. The intensity of fermentation activity was not the same in all locations in the silo, as was indicated by the pH and titratable acidity values as well as by the irregular nature of some of the plate and spore counts. These counts did not always show typical, progressive populations. A greater lack of uniformity among samples would be expected in a more or less solid medium like the silage than in a fluid medium.

MICROSCOPIC COUNTS

The microscopic counts of individual Gram-positive cells give a reasonably good picture of the progress of the fermentation with respect to bacterial populations. In some ways it appears to give a better insight into the nature of this type of fermentation than do the cultural methods. Since fermentation activity did not always progress at the same rate in all locations in the ensiled mass, the sequence of microscopic fields was selected principally on the basis of the stage of fermentation of individual silage samples and not with strict regard to fermentation age of the particular sample (fig. 3).

The microscopic study revealed that the predominating flora was wholly Gram-positive and divided more or less into two morphological types; a short thick rod and a rather long rod of medium thickness (fig. 3, *C* and *D*). There is a possibility that a third type occurred, a much smaller rod than the others. Both principal types tended to elongate and form long chains and filaments in the more acid samples taken during the latter part of the fermentation (fig. 3, *G*). Predominating colonies picked from the routine platings likewise fell into the above morphological groups and showed the same tendency toward elongation in old cultures of broth media that had reached maximum acid production.

FIGURE 3.—Photomicrographs of the predominating micro-organisms present during the acid fermentation of steamed potato silage. All×1700. Slides used were those prepared and stained for the microscopic cell counts of Gram-positive cells. *A*, *B*, Vegetative cells from samples taken during the early stage (10 and 16 days) of fermentation; dark area in background of *B* is a particle of steamed potato tissue. *C*, *D*, Samples taken at 33 and 50 days; both showing active acid fermentation with large populations of short, thick vegetative rods and a few of the more slender types. *E*, *F*, Samples taken at 21 and 42 days showing more advanced stage of fermentation and evidence of active spore formation; note clostridial form (*E*, *a*) and free, oval type spores (*F*, *a*). The background in each case (*E*, *F*) is composed of indistinct dead cells. *G*, Elongated cell type that predominated during the latter part of the fermentation; note definite swollen condition of one end (*a*) of the cell. Such cells gave rise to small spherical spores rather than to the large oval types shown in *F*, *a*. *H*, Mass of entangled vegetative cells at the edge of a particle of potato tissue; growth appears to channel into the tissue (at right); silage sample 42 days old.

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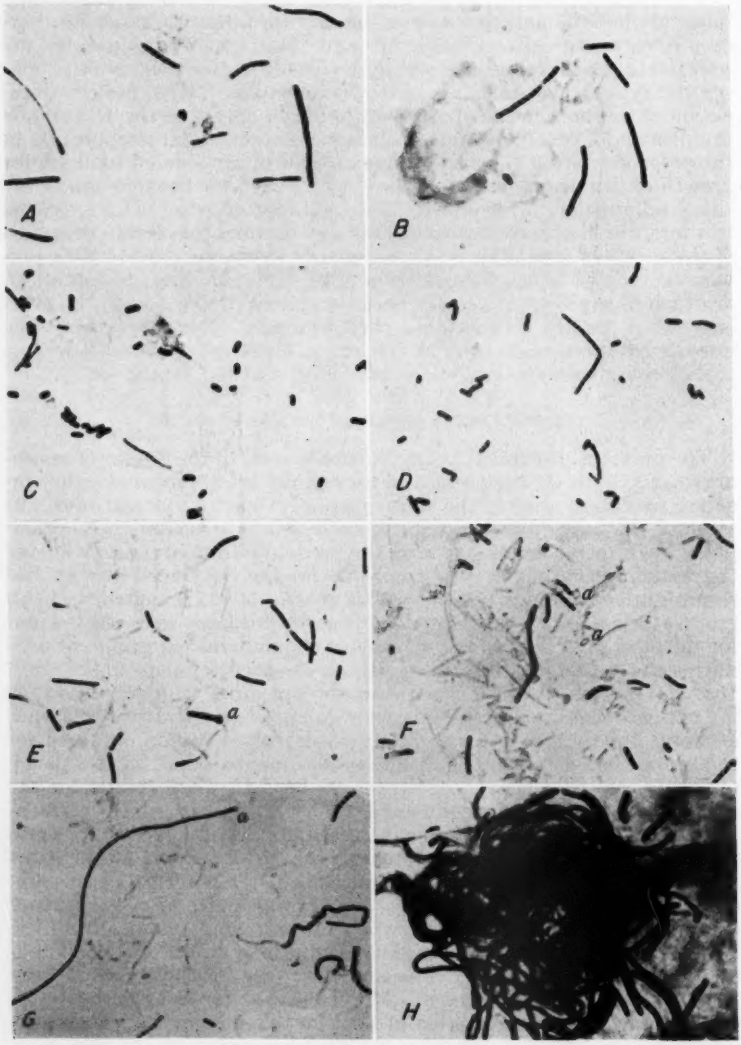

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The spore counts reveal that active spore formation took place during the fermentation, which would suggest that the principal organisms involved were sporulating types. This view gains further support from the fact that all principal colonies isolated from routine plates proved to be Gram-positive spore formers. This is not surprising in view of the degree of heat resistance required by any group surviving both the steaming operation and the period of elevated temperature of the mass during the first 12 days in the silo. The counts further showed that the individual spores fell into two general classes, those that were spherical, and those that were oval (fig. 3, *F*). There seemed to be at least two distinct sizes of oval spores. The majority of the spores counted were free from the cells.

CLASSIFICATION OF THE PREDOMINATING GROUP

During the active fermentation period, a number of isolates representative of each predominating colonial type were made from the dextrose tryptone agar and nutritive caseinate agar routine plates. Thus far sufficient work has not been done to assure identification other than classification within the genus *Bacillus* (Cohn) as thermophiles in group X, as listed by Bergey et al. (*1*). Furthermore, they are nongas-producing, spore-bearing, acid-producing rods, which are facultative with respect to oxygen and temperature requirements. One culture in particular is of interest in that it does not appear to ferment dextrose broth or give visible growth on dextrose or plain agar slants at either 35° or 55° C. However, excellent growth and acid production are obtained in liver broth and in liver agar stabs at both of these temperatures.

In general, liver broth and liver agar were more satisfactory than other media for the cultivation of the cultures isolated from the fermentation. Also, during the latter part of the routine platings, plain liver agar was used in conjunction with the routine media for 55° and 35° C. counts with superior results. With the few trials that were made, liver agar plus bromcresol purple gave higher total counts, larger colonies, and faster growth than the routine media used in this study.

QUALITY OF THE SILAGE

At each sampling period the potatoes were examined to determine the general silage quality. The core samples were all judged good and had a characteristic aromatic odor, and acid taste, and firm texture. The texture resembled that of a boiled potato when cold. The ensiled material formed a more or less solid mass of potatoes pressed tightly together and was free from channels and gas pockets (fig. 1, *G* and *H*). It was not mushy, and no free liquid was present. The aromatic nature of the silage appeared to become stronger with the age of the sample and suggested the presence of a mixture of alcohols, organic acids, and esters. Furthermore, the older samples gave some indication of starch hydrolysis or other structural changes not present in those taken earlier. There was no appreciable color change of the potatoes resulting from the fermentation. The surface layer of potatoes, directly underneath the paper covering was considered spoiled since it had an unpleasant odor and soft texture.

SUMMARY AND CONCLUSIONS

The results of a bacteriological examination of the fermentation of hot-ensiled steamed potatoes (*Solanum tuberosum*) have been presented. The bacteriological findings indicated that the thermophilic, facultative anaerobes were the predominating micro-organisms during the fermentation and were responsible for the developed acidity and resultant preservation of the silage. They are considered to be non-gas-producing, acid-forming, spore-bearing rods, which are facultative with respect to oxygen and temperature requirements. These organisms may be classified according to Bergey et al. as thermophiles belonging in group X of the genus *Bacillus*.

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